AZYGOZYGUM CHLAMYDOSPORUM NOV. GEN. ET SP. A PHYCOMYCETE ASSOCIATED WITH A DISEASED CONDITION OF *ANTIRRHINUM MAJUS*

BY

CHAS. G. C. CHESTERS

REPRINTED FROM THE

TRANSACTIONS OF THE BRITISH MYCOLOGICAL SOCIETY

VOL. XVIII, PART III, DECEMBER 1933

CAMBRIDGE

AT THE UNIVERSITY PRESS

PRINTED IN GREAT BRITAIN

[Reprinted from the
TRANSACTIONS OF THE BRITISH MYCOLOGICAL SOCIETY,
Vol. XVIII, Part III, 1933.]

AZYGOZYGUM CHLAMYDOSPORUM NOV. GEN. ET SP. A PHYCOMYCETE ASSOCIATED WITH A DISEASED CONDITION OF *ANTIRRHINUM MAJUS*

By CHAS. G. C. CHESTERS
(*Department of Botany, The University, Birmingham*)

(With Plate XI and 4 Text-figures)

Introduction

The Phycomycete which is the subject of this paper was obtained in culture during attempts to isolate the organism causing a collar-rot and foot-rot of *Antirrhinum majus* (Pl. XI, fig. 1). The development of the perfect spore is in many respects different from that described for other Phycomycetes, and it has been impossible to place the fungus in direct relation to any described group. The early stages, which are initiated by the fusion of two hyphal branches, suggest an affinity with the Zygomycetes, but the later stages diverge from those so far described for fungi belonging to that group. The absence of a sporangial or conidial stage and the continual reproduction by means of chlamydospores render it difficult to assign a definite position to this fungus. Growth on a variety of media and under diverse conditions has failed so far to induce the formation of any other spore form. The perfect spore appears to be non-functional under cultural conditions and degeneration of its contents often occurs.

The fungus was present in the cortex of diseased plants and was associated with the actual pathogen which in some cases was *Rhizoctonia Solani* Kühn and in others *Phyllosticta Antirrhini* Syd. The seedlings affected were growing in cold frames. On the surface of diseased stems and especially on the roots numerous intercalary chlamydospores ornamented with short blunt spines were produced. These were also found in diseased soil in contact with the seedlings. They were spherical and measured from 10 to 26μ in diameter (Pl. XI, fig. 2). In one specimen large hyaline thick-walled spores about twice the size of the chlamydospores were observed (Pl. XI, fig. 2, bottom right-hand corner). Both spore forms have been developed in culture.

These chlamydospores were isolated and germinated on malt agar. The mycelium produced gave rise to chlamydospores of similar shape and size within a few days. On this medium and on several others the second spore form was later developed on single chlamydospore cultures. This spore was usually a bicellular structure consisting of

a thick-walled, hyaline or yellowish upper cell and a thinner-walled tapering stalk attaching it to the mycelium (Pl. XI, fig. 4 D). The thick-walled cell contained a single large oil drop occupying the major portion of the cell which was almost spherical and measured from 30 to 36μ in diameter. Such spores were also produced in sterilised garden soil after about fourteen days' growth.

Experimental methods

Of the several media used during this investigation the most satisfactory have proved to be Ashby's modification of Sherbakoff's maize agar(1), dilute malt-extract agar containing 2 per cent. of maltose sugar—determined by titration—and nutrient sucrose-nitrate agar containing 20 gm. sucrose, 2 gm. KNO_3, 0·5 gm. $MgSO_4\ 7H_2O$, 0·5 gm. KH_2PO_4, 30 gm. agar per litre of distilled water.

Thin films of mycelium which could be fixed and stained without disturbing the arrangement of the hyphae were obtained by placing sterile glass slides in plates of maize agar. Such preparations stained with cotton blue in lacto-phenol-glycerine (Amann's medium) were used to study the development of the two spore forms.

The development of individual thick-walled spores over a considerable period was followed by using a modification of a method suggested by Henrici (2), in which a cell of uniform depth was prepared by supporting a $2 \times \frac{7}{8}$ in. cover-glass on a microscope slide by means of three glass strips. These were cemented to the slide and cover-glass by beeswax and resin mixture. Such a cell, when filled with agar and inoculated, allowed observation of any particular portion of the mycelium and with ordinary care remained free from contamination for several days.

Material for cytological or detailed morphological study was fixed in Flemming's weak solution for one hour and then transferred to 1 per cent. chromic-acetic for twelve hours, washed in water for twenty-four hours and brought up to absolute alcohol through grades of 10 per cent. Microtome material was embedded in wax by the chloroform method or in collodion and then in wax. Sections were cut at 5 and 10μ and stained either in haematoxylin or cotton blue. The haematoxylin was prepared as a 0·5 per cent. solution in 50 per cent. alcohol and used after mordanting with a 0·25 per cent. solution of iron alum in 50 per cent. alcohol. A half-saturated solution of eosin in 50 per cent. alcohol was used as a counter-stain. At times half-strength Delafield's haematoxylin was employed and the sections were differentiated, after overstaining, in acid alcohol. The cotton-blue stain was prepared by dissolving 0·5 per cent. of cotton blue in Amann's medium (3). After staining for twenty-four hours the sections were differentiated in the lacto-phenol-glycerine mixture, washed in

95 per cent. alcohol, dehydrated, cleared and mounted in balsam. For general cytoplasmic structure this proved a useful and reliable stain, especially when examining hyphae embedded in plant tissue, since only the hyphae and their contents were stained.

The Phycomycete was isolated from several varieties of *Antirrhinum*, but only the strains from Beauty, Dazzler, Afterglow, Crimson Bedding and Appleby Matthews were retained for study. Isolations were made both from chlamydospores detached from the mycelium and from hyphae in infected tissue. Standard malt agar of *p*H 6·5 was used in making the isolations and for maintaining the stock cultures. Each stock culture originated from a single chlamydospore taken from an isolation plate.

Life history

(1) *The mycelium.* When chlamydospores are sown on malt agar and incubated at 24° C. each spore produces one or more long germ-tubes during the first eight to ten hours. These soon commence to branch and a small radial colony is established within twelve to fifteen hours. The hyphae at this stage are irregularly swollen and bead-like. These swellings are characteristic of the mycelium at all stages of its development (Text-fig. 4 A, H and J). On media containing nitrate or peptone they are very marked and each swelling is densely filled with oil drops. The ultimate branches of the immersed mycelium generally arise from such swellings and are very finely attenuated, especially on maize agar. Within the cortex of sterilised *Antirrhinum* stems the swellings are much exaggerated. Beginning as an irregularly enlarged branch (Text-fig. 4 B and C) numerous globular swellings are formed over its surface. From each of these a hypha is formed which may itself be tapered or may give rise to a series of attenuated branches (Text-fig. 4 D) which penetrate the cortical cells in all directions. These large masses of filaments have been observed only in culture.

The mature hyphae contain rather granular protoplasm in which small nuclei are embedded. At first the protoplasm is dense, but later it assumes a reticulate appearance due to the presence of numerous vacuoles (Text-fig. 4 A). The oldest hyphae are filled with oil drops and septa occur at intervals.

(2) *The chlamydospores.* From thirty-six to forty hours after germination has begun mature chlamydospores are present on the mycelium. These generally arise on small lateral branches but may occur on the larger branches. On small lateral branches only one chlamydospore is usually formed (Text-fig. 3 A, B and E). Although these are typical chlamydospores in the accepted meaning of the term they differ from those described for most Phycomycetes in that they do not appear

at a late stage in development when the hyphae are undergoing degeneration but are formed early in the life of the mycelium. They have a double wall and may be covered with short blunt spines which are outgrowths of the outer wall (Text-figs. 1 A and 3 E). When the chlamydospores are produced in moist air or in water the spines are longer and sharper than those produced on superficial or immersed spores. They are perfectly spherical and measure from 10 to 26μ in diameter. They have numerous small nuclei (Text-fig. 3 C–E) and the density of their protoplasm gives them a dark appearance when young, but they are not pigmented and, as they age, oil drops accumulate in their protoplasm. Their production continues throughout the life of the mycelium. The immersed forms are ovoid or slightly irregular and may often have no spines and a comparatively thick wall (Text-fig. 1 C and D). In old cultures portions of the hyphae are simply divided off into rather thick-walled segments (Text-fig. 1 E and F). These are more akin to the chlamydospores of other Phycomycetes.

(3) *The thick-walled spores.* While the chlamydospores appear soon after germination the other spore form does not reach its adult state until some five to fifteen days have elapsed. These spores occur either on the surface or immersed hyphae.

Maize and sucrose-nitrate agars were employed to study the details of the development of the second spore form. This spore is initiated at about the same time on all media but earliest on sucrose-nitrate agar, due, no doubt, to a certain lack of available food materials which gives rise to a sparse vegetative growth, and latest on malt agar on which abundant vegetative growth occurs. The spore is always initiated by the fusion of two hyphae (Text-fig. 2 A–C). These are usually two small lateral branches (Text-fig. 2 A and C), but often the end of a small branch fuses with an undifferentiated portion of another branch. In either case no active growing together of the fusion hyphae has been observed. On the contrary it would appear that in the first instance the branches must be touching. The two branches may arise from the same (Text-fig. 2 A) or from different parent branches (Text-fig. 2 B–F). Even in the youngest condition the two hyphae may be distinguished from one another in size. The larger is destined to become the stalk of the thick-walled spore. Independent of the type of medium on which they develop, a difference of structure becomes evident about the time when the branches, having fused, begin to enlarge. The smaller branch is always more hyaline than the larger (Text-fig. 2 D–F), and its protoplasm appears reticulate in structure. At this time the rudiment is distinctive, consisting of the two swollen ends of the attached hyphae and their thinner basal portions. These basal parts may branch to a varying degree. On maize agar very numerous tapering branches are formed,

some being of considerable length, on malt fewer and shorter branches occur, while on sucrose-nitrate agar there are practically no branches at all. As the two hyphae increase in size the smaller loses its hyaline and reticulate structure and becomes more or less granular (Text-fig. 2 E). It increases in size more rapidly than the larger and often the contents of the stalk become depleted in the process. When the two terminal portions are about the same size a septum is formed cutting off the originally smaller portion from its stalk (Pl. XI, fig. 4 A), which then degenerates entirely except sometimes for a minute tail above the septum. The stalk of the other branch remains attached and becomes thicker towards the swollen upper part. The walls of both parts thicken, oil appears in the protoplasm and the whole contents become dense and somewhat brown. Subsequent development may take place in either of two ways. Either the wall separating the two attached branches may become absorbed in part or completely, or the wall may remain and that of the terminal cell increase in thickness.

The first type of development occurs relatively rarely. It is a condition characteristic of a certain degree of maturation. Dissolution of the dividing wall only takes place after the deposition of secondary wall material has begun in either or both attached hyphae. This wall may break down towards the centre (Pl. XI, fig. 3 A) and later throughout its length, or several pores may appear (Pl. XI, fig. 3 B) and islands of the wall become separated. A thick wall is formed around the combined protoplasts, in which oil has begun to accumulate. The spherical or pear-shaped fusion cell is still attached to the mycelium by a single well-defined stalk.

Such large fusion cells have been kept under observation for several months, and a few have been isolated from the mycelium, but under various experimental conditions no germination has been observed. While attached to the mycelium they either do not change in appearance—except for a slight yellowing of the wall—or the wall becomes greatly thickened and partially closes the pore between the two parts (Pl. XI, fig. 3 C and D). In the latter condition oil rapidly accumulates and the protoplasm becomes disorganised.

The second course of development is the more usual. The contents of the terminal cell aggregate towards its centre forming a dense zone surrounded by a lighter area in which oil drops appear. Later the central mass becomes filled with oil drops (Pl. XI, fig. 4 B) which increase in size and coalesce (Pl. XI, fig. 4 C) until a single large globule is formed enclosed by an almost clear peripheral zone (Pl. XI, fig. 4 D). During this time the wall has increased in thickness. The basal cell, attached to the mycelium, retains its granular structure for a longer period (Pl. XI, fig. 4 D), but in time it also undergoes a similar series of changes. Often the whole contents of this cell degenerate and the terminal cell is set free.

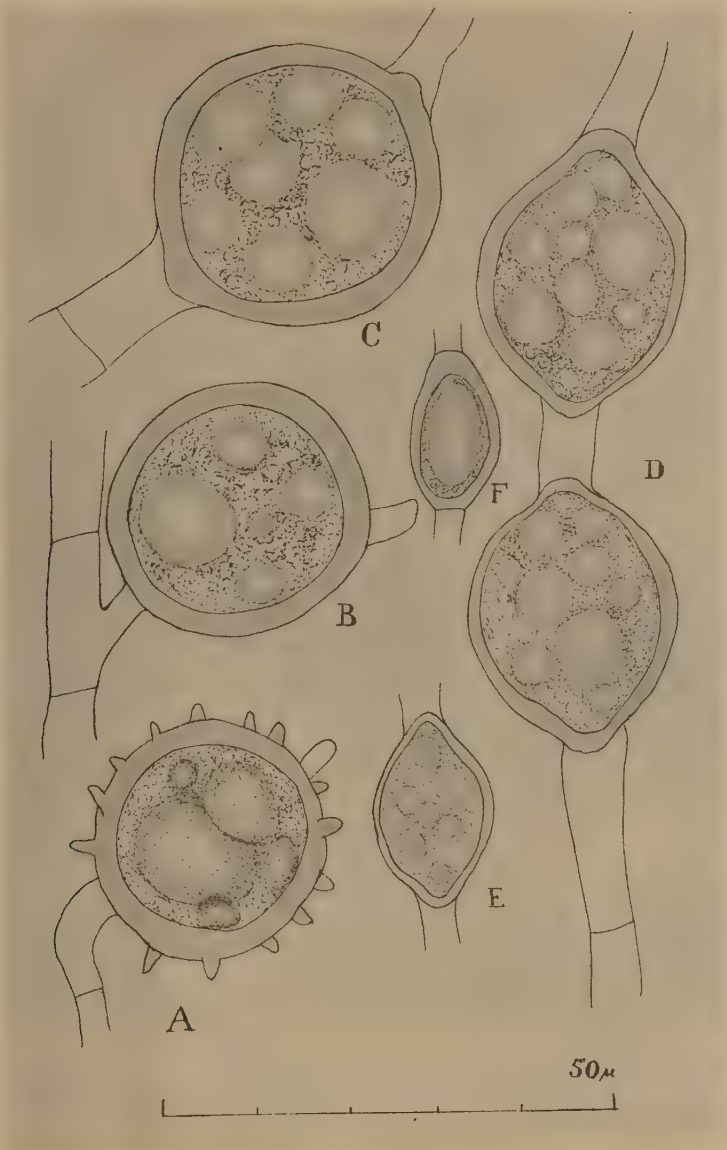
The cytological examination of such stages in the development of the pairs of attached hyphae, as have been described above, merely confirms the fact that any communication which is established between the two elements is formed at a comparatively late stage.

The earliest stages examined exhibit that differentiation in the size and character of the cell contents of the two hyphae which has already been noted (Text-fig. 3 F–H), and in progressively older stages the gradual increase in the size of these parts is observed (Text-fig. 3 J) until both elements are of approximately equal dimensions (Text-fig. 3 K). Up to this point the walls of the two hyphae have remained thin, and numerous small nuclei are fairly distinct. Very little oil is present in the two structures. Further increase in size takes place, oil appears in the contents of the cells and the walls begin to thicken (Text-fig. 3 L) in such a manner that a three-layered wall is formed. Each layer gives a distinct staining reaction. The outer is yellowish and does not stain easily or permanently; the middle layer, which varies in breadth, stains densely with haematoxylin; and the inner, comparatively thick layer, stains pale pink or salmon colour with eosin or congo red. This latter layer may increase in thickness during later stages of development.

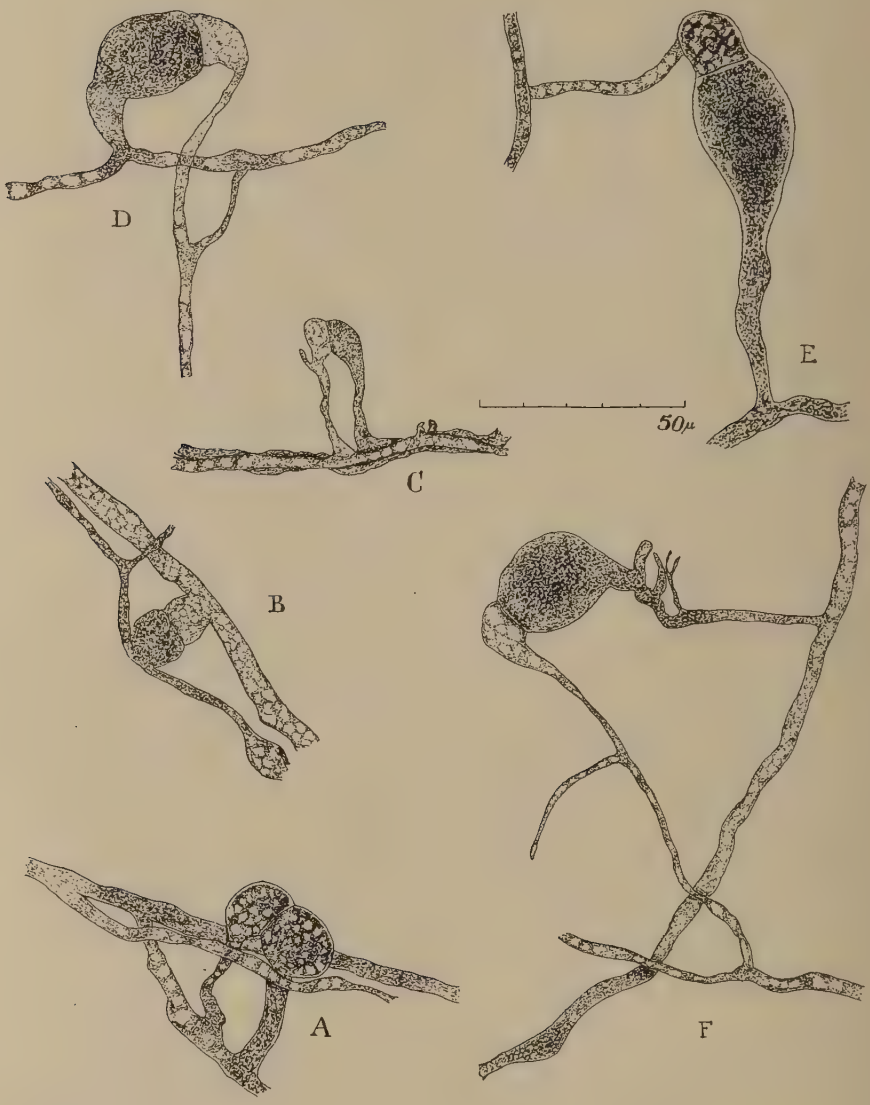
Where open communication is established between the two attached cells the breakdown of the dividing wall is initiated by changes occurring in the central layer which begins to swell up at one or more places throughout its length (Text-fig. 3 L). These swellings increase in size and one or more small pores appear in the wall (Text-fig. 3 M). When only a single pore is formed this becomes enlarged, but when more than one occurs islands of wall material are separated and lie embedded in the protoplasm of the cell or cling to the wall (Text-fig. 3 O). The large fusion cell (Text-fig. 3 P) has numerous nuclei and a prominent and fairly thick inner wall which extends throughout the cell. The remains of the dividing wall are still visible (Text-fig. 3 P).

Where communication fails to be established between the two cells there is no visible swelling of the middle layer of the wall (Text-fig. 3 N), but a small pore may penetrate the inner wall. The contents of the two cells become replaced by oil which ultimately occupies the centre of each cell and is surrounded by a mass of degenerate protoplasm. The basal cell may lose its contents. The majority of the spores examined show that abnormal development of the innermost layer of the wall initiates the degenerative processes.

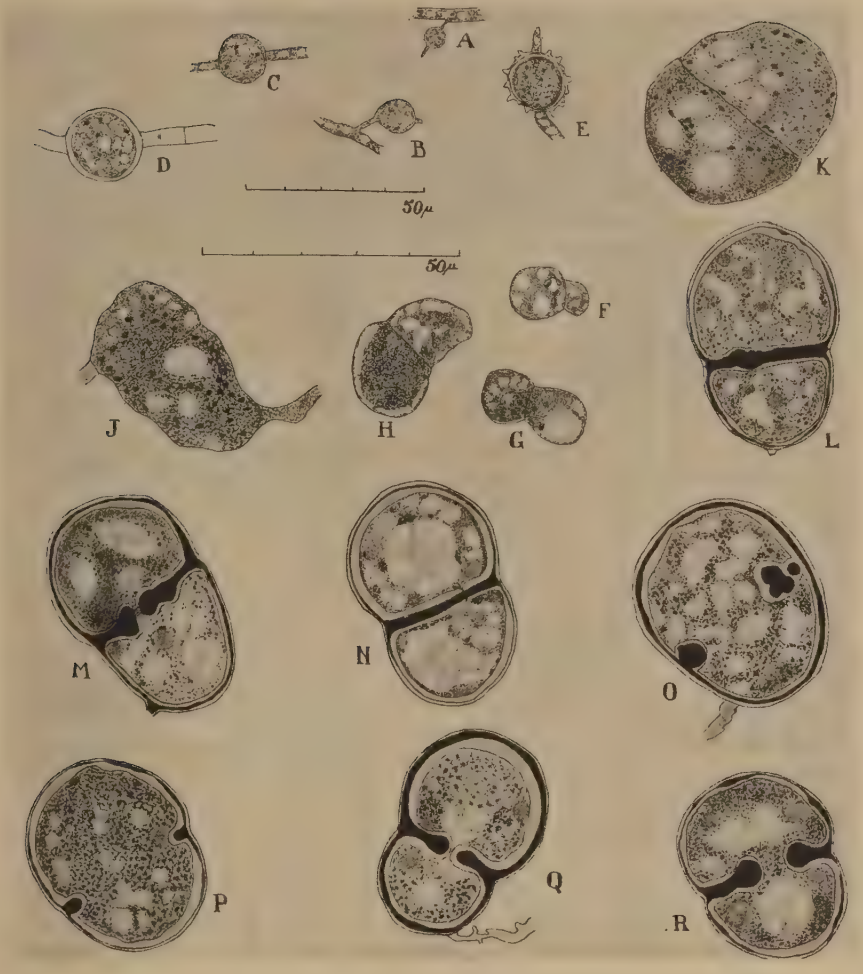
A proportion of those in which a fusion cell is formed appear to degenerate. This condition exists when the pore is established after much thickening of the inner cell layer has occurred and when a very localised swelling of the middle layer takes place (Text-fig. 3 Q and R). Here the contents quickly degenerate and are replaced by oil, and



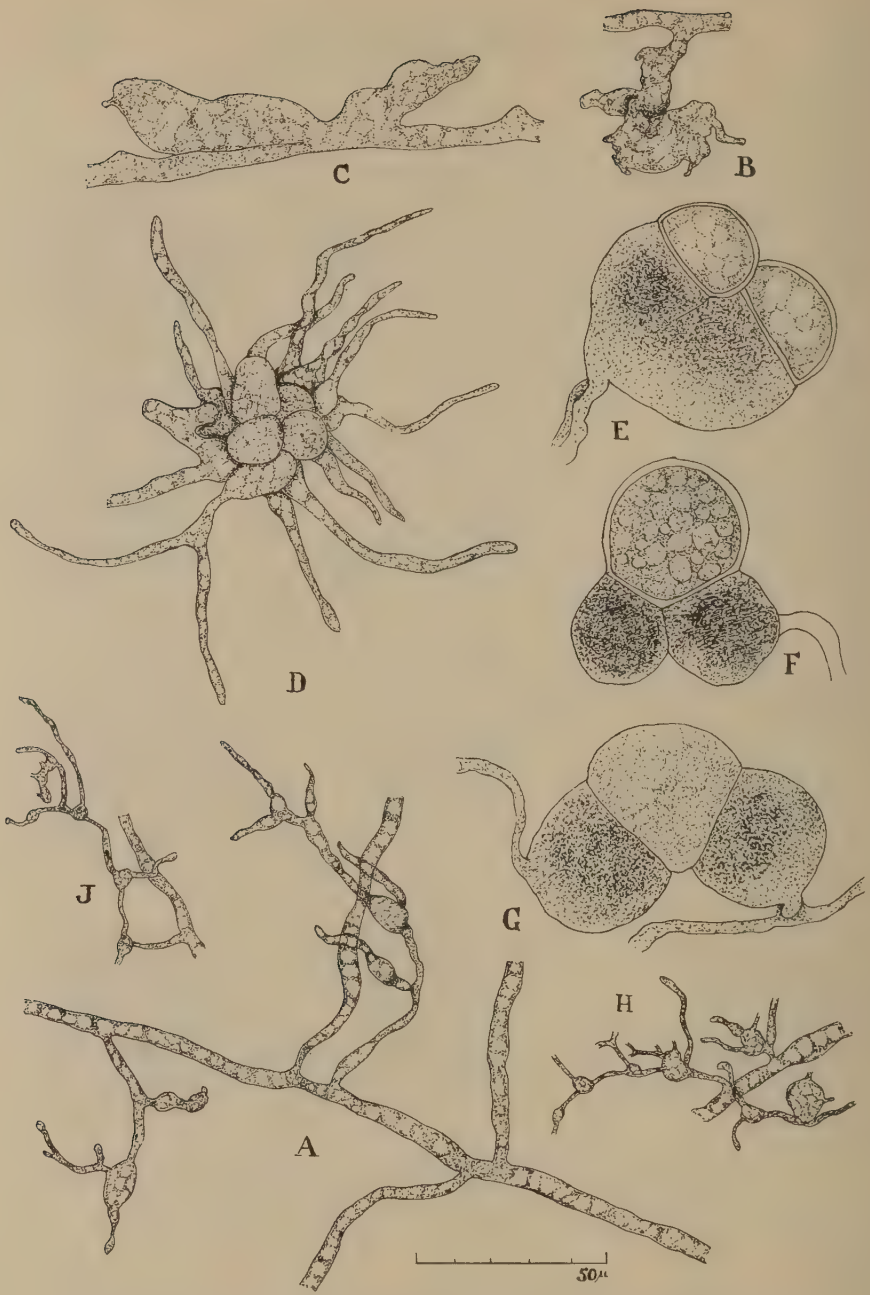
Text-fig. 1. Chlamydospores. A, from aerial mycelium on malt agar; B, from immersed mycelium; C–F, from deeply immersed parts of the mycelium of an old culture on malt agar.



Text-fig. 2. Young fusion hyphae. A–E, from sucrose-nitrate agar; F, from maize agar.



Text-fig. 3. Sections of various stages in the development of the chlamydospore and the thick-walled spore. A–E, development of the chlamydospore; F–K, early stages in the development of the thick-walled spore; L–R, later stages of the same.



Text-fig. 4. Appearance of the mycelium. A, H and J, in malt agar; B, C and D, in *Antirrhinum* stems; E–G, certain fusion hyphae (for further explanation see text).

the inner wall thickens to such an extent as almost to close the pore (cf. Pl. XI, fig. 3 C and D).

(4) *Cultural characteristics.* The growth characteristics of the fungus are so clearly defined on all media that a summary of them seems fitting. The chief results of the cultural treatments given below are summarised in the table.

Media used	Aerial mycelium	Surface mycelium	Submerged mycelium	Chlamydospores	Resting spores	Comparative growth
(1)	White dense zoned	Thick zoned	Numerous groups round inoculum	Aerial surface submerged	Surface and submerged	Dense, zoned growth
(2)	White fluffy	Fairly thick	Groups spreading from inoculum	Aerial surface submerged	Mainly submerged	Good growth
(3)	None	Thin	A little but irregular	Surface	None	Sparse growth
(4)	White fairly dense	Very thick	Thin over all cultures	Aerial	None	Dense, zoned; liquifies gelatine in 10–14 days
(5)	Hardly any	Thin spreading	If present—as groups	Aerial surface submerged	Mainly submerged	Fair growth
(6)	Small amount	Fairly thick	A few groups	Aerial surface submerged	None	Good growth, slightly staling
(7)	White fluffy	Thick	Thick over all culture	Aerial surface	None	Dense growth, staling
(8)	Very thin	Thin spreading	Dense and deep	Aerial surface submerged	Submerged	Mostly embedded in the tissues
(9)	Hardly any	Thick	General	A few on surface	None	Fair
(10)	Hardly any	Thick	General	A few on surface	None	About twice as much as on (9)

Media used

(1) Malt extract containing 2 per cent. maltose, 3 per cent. agar.
(2) Maize agar (Ashby's formula(1)).
(3) Potato agar (Brown's formula(4)).
(4) Malt extract gelatine, 12 per cent. gelatine.
(5) Synthetic medium (see p. 200) + 0·2 per cent. KNO_3.
(6) Synthetic medium (see p. 200) + 0·2 per cent. peptone.
(7) Synthetic medium (see p. 200) + 0·4 per cent. peptone.
(8) Steam sterilised *Antirrhinum* stems.
(9) Synthetic medium + 2 per cent. (vol.) olive oil.
(10) Synthetic medium + 2 per cent. (vol.) castor oil.

On any synthetic medium the greatest growth is obtained with maltose as the source of carbohydrate and KNO_3 as the source of nitrogen. Substituting glucose for maltose the amount of growth is almost the same but less aerial mycelium is formed. Sucrose is a

poorer source of carbohydrate. When an organic source of nitrogen is substituted for KNO_3 staling and abnormal development of the mycelium occur in proportion to the concentration of the substance used.

The fungus grows abundantly as a saprophyte in ordinary garden soil, producing both spore forms. When *Antirrhinum* seed is sown in such infected soil the seedlings produced remain comparatively healthy and no clear case of parasitism by the fungus has so far been observed.

Discussion

There is no doubt that this fungus must be placed in the Phycomycetes. The eseptate mycelium consisting of rather coarse hyphae filled with granular protoplasm and numerous small nuclei is characteristic. This mycelium bears both chlamydospores and large thick-walled spores which arise by the fusion of two differentiated hyphal branches. Its exact position will be determined by the interpretation placed on the character of the mycelium, the thick-walled spores and the chlamydospores.

(1) *The mycelium.* The mycelium is extensive whether it occurs on diseased *Antirrhinum* roots, in infected soil or in culture on various media. This indicates a relationship with the higher rather than with the lower Phycomycetes. Within the Chytridiales, as they are delimited by Fitzpatrick(5), the mycelium, if present, is poorly developed. Recently a new genus of the Cladochytriaceae has been described by Sideris(6) under the name *Rhizidiocystis*. The only species, *R. Ananasi*, has an extensive mycelium parasitic on pineapple roots. In general appearance it bears a similarity to the fungus on *Antirrhinum*. Both develop on roots and produce a fluffy white mycelium. The hypnospores of *Rhizidiocystis* and the chlamydospores of this fungus are of somewhat similar shape and size. The former fungus produces zoosporangia. Such structures have never been observed during the course of the present investigation. *Rhizidiocystis* has an evanescent mycelium, while this has a persistent mycelium. Any similarity is superficial, and there is no doubt that this fungus should be placed in a higher order.

(2) *The thick-walled spore.* The absence of a motile stage in any part of the life history suggests a position near to the Zygomycetes, and this is supported by the development of the thick-walled spore. The value to be placed on these developmental stages can be assessed only after the following features have been considered. The fundaments of the thick-walled spore are two attached, disproportionate hyphae. The originally smaller hypha becomes abstricted from the mycelium after a period of growth. Communication between the two parts may never be established, and the whole structure may become bicellular

with a thick-walled distal cell. Communication may occur and a thick-walled fusion cell be formed. The formation of a fusion cell is a late phenomenon.

In all described Zygomycetes the hyphae which fuse to give the zygospore are recognised as progametangia, each of which later becomes differentiated into a basal persistent suspensor and a terminal gametangium. Only the two gametangia take part in the production of the zygospore whose thickened wall is laid down within that of the fused gametangia. At maturity the zygospore remains attached to both suspensors.

This Phycomycete shows no differentiation of the attached hyphal branches unless it be considered that the tapering basal parts of the two branches represent suspensors, and, even if this be so, one of these is not persistent. Although the earliest stages in the formation of the thick-walled spore are reminiscent of zygospore production subsequent development proceeds along different lines, and either a bicellular structure, in which the upper portion becomes spore-like, or a large fusion cell, attached basally, is formed. The former condition has no parallel in the Zygomycetes. Azygospores are known in several genera under certain cultural conditions, but these always arise from one gametangium and not, as here, from an isolated part of one hypha attached to another. The latter condition is also atypical. It occurs after thickening of the cell wall of the two elements has taken place and not, as in the zygospore, while the fused hyphae are still young. Also, the wall of a zygospore is a new structure formed around the fused protoplasts, while here the thick wall is merely an extension of a structure already present.

The occurrence of spores in which an extremely small pore is formed or in which increase in the depth of the inner wall almost occludes the pore indicates that the whole process is extremely unstable. This is emphasised by a very few observations of young, apparently typical zygospores (Text-fig. 4 G) formed between swollen parts of two fused hyphae. These were produced on a sucrose-nitrate culture, but attempts to repeat their formation have so far failed. On malt agar others were observed (Text-fig. 4 E and F) in which either the basal or the distal parts of similar structures were fused together. These three examples are interpreted as indicating that the fusion elements seen in all cultures are really zygospore-like rudiments, but that for some reason development is abortive. The cause of this does not appear to lie in the fact that the fungus is heterothallic, as all isolations behave alike and when grown against one another fail to produce zygospores but do produce thick-walled spores.

I am of the opinion that the initial stages in the development of the second spore form of this fungus can be legitimately compared with

the initial stages in zygospore production, and on that account place the fungus in the Zygomycetes. Later development is regarded as abortive, and the special instances illustrated in Text-fig. 4 are thought to indicate that, if correct conditions were established, normal zygospores would be produced.

(3) *The chlamydospores.* These structures are produced on almost every medium, and, if the fungus has been recorded previously, at least the chlamydospores should have been described. They are always intercalary, sometimes singly, sometimes in series. Spines, which are outgrowths of the outer wall, occur when the chlamydospores are aerial or are formed in water. Transitional stages exist between these aerial chlamydospores and others formed from thick-walled mycelial segments which occur immersed in the substrate.

A search through available literature has failed to discover any imperfect genus having chlamydospores of the type described. *Chaetoconidium* possesses chlamydospores which are somewhat similar, but they are produced on a septate mycelium (7). The genus *Humicola* was created by Traaen for two soil fungi having chlamydospores (8)*. There is general resemblance to *H. grisea* but there are marked differences. The chlamydospores of the latter are usually solitary, seldom in chains and never regularly intercalary. They are apparently not ornamented and the mycelium is brown or dark coloured.

Within the Zygomycetes several genera produce chlamydospores, and in *Mortierella* those on the aerial mycelium are specialised (stylospores). They resemble the aerial chlamydospores of this fungus in size and in the fact that they are ornamented, but they are generally produced singly upon short upright branches. The development of the stylospores of *M. polycephala* has been described by Dauphin ((9) p. 41 *et seq.*). The spore (*loc. cit.* Fig. 33) is remarkably similar to the chlamydospore of this species except that it is not an intercalary structure. Dixon-Stewart (10) has described a new species of *Mortierella* —*M. stylospora*—which was isolated from garden soil. She states that: "Reproduction in culture appears to be by means of well-developed stylospores." The latter are produced on upright aerial hyphae 40μ in length and 18μ in diameter. Rudimentary zygospore masses are also formed, but the zygospore does not mature in culture.

As far as the chlamydospores are concerned this Phycomycete is more closely related to *Mortierella* than to any other genus of the Zygomycetes, but the affinity, if it exists, cannot be stressed as zygospores of a type similar to *Mortierella* have not been observed. It must be realised also that the chlamydospores of a fungus are not always a reliable criterion of its relationship. The branching of the basal parts of the fusion hyphae (cf. p. 202 and Text-fig. 2 F) may not

* I am indebted to Mr Ramsbottom for bringing this genus to my notice and for supplying an abstract of the original description.

be without significance, as it is from the suspensors that the hyphae investing the zygospore of *Mortierella* are produced (9).

It is not intended to suggest that this Phycomycete is a species of *Mortierella*, but it is considered that its position in the Zygomycetes is certainly in close proximity to the Mortierellaceae.

Azygozygum nov.gen.

Mycelium effusum, persistens; hyphae eseptatae, ramis alternis, ramulis ultimis exquisite attenuatis.

Chlamydosporae intercalares, solitariae v. seriatae, sphericae v. ellipsoideae, cortici v. substrato immersae, episporio laevi instructae, superficiales spinis ornatae.

Sporae vulgo abortivae, bicellulares, cellula terminali laevi crassitunicata, basali tenuiter tunicata, inferne attenuata; at maturae zygosporicae, crassitunicatae, laeves, ferme sphaericae, mycelio basi adnatae.

Sporangia aut conidia nulla visa.

Habitat ad quisquilias putridas in horti solo jacentes.

A. chlamydosporum nov.sp.

Mycelium effusum, persistens, album; hyphae eseptatae, oleo copioso farctae, irregulariter tumefactae, ramis alternis, 3–5μ diam., ramulis ultimis attenuatis, ex hypharum tumoribus oriundis.

Chlamydosporae numerosissimae, intercalares, solitariae v. seriatae, superficiales v. immersae; superficiales achroae v. lutescentes, sphaericae, 10–26μ diam., spinis obtusis praeditae, in matrice immersae ellipsoideae, laeves, crassitunicatae, luteolae.

Sporae vulgo abortivae, bicellulares, cellula terminali crassitunicata, laevi, luteola, ferme sphaerica, 30–36μ diam., basali tenuitunicata, achroa, magnitudinis aequalis, inferne attenuata; at maturae zygosporicae, crassitunicatae, laeves, luteolae, fere sphaericae, 40–60μ diam., achroae v. lutescentes, mycelio basi adnatae.

Plerumque matrici immersum est. Ex *Antirrhini* stipitibus radicibusque, *Rhizoctonia Solani* Kühn affectis, aeque ac ex horti solo prope Birmingham Angliae excerpsi et arte colui.

Summary

1. The fungus was found associated with a diseased condition of *Antirrhinum majus* due to *Rhizoctonia Solani* and at times to *Phyllosticta Antirrhini*.

2. The appearance of the spiny chlamydospores attached to eseptate mycelium on the diseased plants and in the soil is described.

3. The organism has been grown in culture.

4. Chlamydospores similar to those on the host are developed in culture. Also a second spore form which may be either a bicellular

structure or a thick-walled fusion cell. These arise by the fusion of two hyphal branches.

5. Neither of the two thick-walled structures has been germinated.
6. Cultural characteristics are dealt with.
7. It has been found impossible, so far as experiments have gone, to induce this fungus to attack living *Antirrhinum* plants.

The writer wishes to acknowledge the kindness of Mr W. B. Grove in translating the diagnoses into Latin, and much helpful criticism received during the course of this investigation from Dr E. J. Butler, Mr S. F. Ashby, and Mr J. Ramsbottom.

EXPLANATION OF PLATE XI

Fig. 1. *Antirrhinum* plants suffering from collar-rot from which the Phycomycete was obtained.
Fig. 2. Chlamydospores and two thick-walled spores in the cortex of diseased *Antirrhinum* stems.
Figs. 3, 4. Stages in the development of the fusion hyphae.

REFERENCES

(1) Wiltshire, S. P. "A method for the preservation of Petri dish cultures of fungi." *Trans. Brit. Myc. Soc.* xv (1930), 93.
(2) Henrici, A. T. *Moulds, Yeasts and Actinomycetes* (1930).
(3) Linder, D. H. *Science*, N.S., lxx (1918), 430.
(4) Brown, W. "Studies in the physiology of parasitism." *Ann. Bot.* xxix (1915), 318.
(5) Fitzpatrick, H. M. *The Lower Fungi, Phycomycetes* (1930).
(6) Sideris, C. P. "*Rhizidiocystis Ananasi* Sideris nov.gen. et sp.—a root-hair parasite of pineapples." *Phytopath.* xix (1929), 367.
(7) Lindau, G., in Rabenhorst, *Krypt.-Flora. Deutsch.* i, viii (1905), 185.
(8) Traaen, A. E. "Untersuchungen über Bodenpilze aus Norwegen." *Nyt magazin for Naturvidenskaberne*, lii (1914), 19.
(9) Dauphin, J. "Contribution a l'étude des Mortierellées." *Ann. Sci. Nat.* (9 ser.), viii (1908), 1–113.
(10) Dixon-Stewart, D. "Species of *Mortierella* isolated from soil." *Trans. Brit. Myc. Soc.* xvii (1932), 208.

Fig. 1

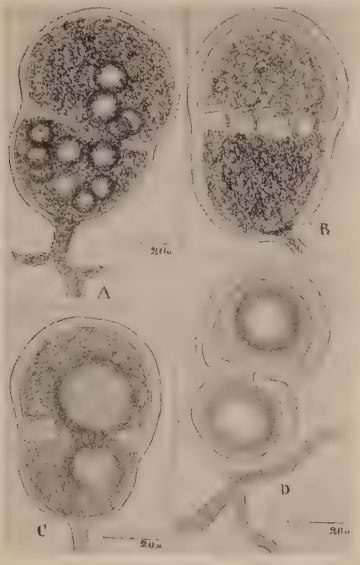


Fig. 3

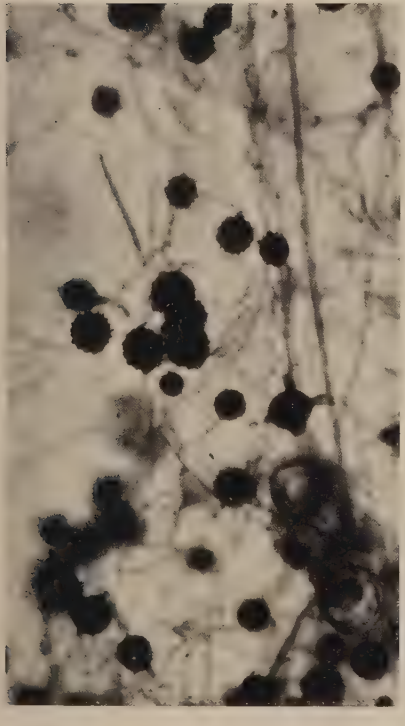


Fig. 2

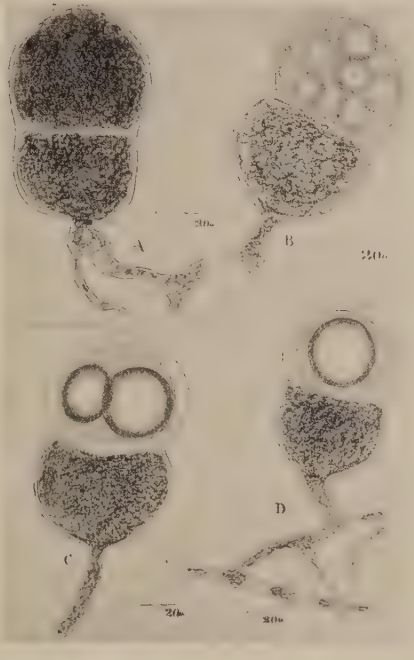


Fig. 4

AZYGOZYGUM CHLAMYDOSPORUM